A

Synopsis of thesis entitled

"Biological control of grey mold disease of tomato (*Solanum lycopersicum. L*) crop by endophytic fungi."

Submitted

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Abstract-

There are increasing efforts aiming to utilize endophytes as biological control agents (BCAs) to improve crop production. Tomato belongs to solanaceae family, and it is one of the most commonly cultivated vegetable crops worldwide. Solanum section lycopersicon includes: the cultivated tomato (*Solanum lycopersicum. L*). Grey mold of tomato caused by *'Botrytis cinerea'* is a highly damaging disease world wide. This study was conducted to identify the endophytic fungi of tomato plant of evaluate their antagonistic effect against the tomato grey mold disease caused by *B. cinerea*,

In this study, endophytic fungi were isolated from the healthy tomato Plants and their ability to control the grey mold disease was investigated in vitro conditions. The results indicate that endophytic fungi have biological control potential and growth promotion by the endophytic fungi.

Keywords : *Solanum lycopersicum. L, Endophytic fungi, Growth promotion, Antagonistic effect.*

Introduction

Gray mold of tomato is one of the most devastating postharvest disease. The disease is present in China from several decades, spreading rapidly and posing a serious threat to tomato production (Wang et al., 2010). Prevention and treatment of this disease is important in order to improve the agricultural products or promote the potential development of tomato industry. The growing public interest in food and environment-related safety issues, and escalated demand for green organic products for healthy living have created an urge for a new, effective, and environmental friendly and biosafety approach to reduce postharvest loss. Biological products based on the beneficial bacteria such as *B. subtilis* L1-21 can be used as an alternative to synthetic fungicides or food preservatives. These products develop a variety of physiological changes in the host plant metabolism, induce systematic resistance (ISR and SAR), and extend the shelf life of fruits (Dimkpa et al. 2009 and Sarma et al. 2012 and Arroyave-Toro et al. 2017). Biocontrol strain of *B. subtilis* WXCDD105 was used to control tomato gray mold involving antagonistic approach but could not give the exact mechanism of disease control (Wang et al. 2018). Previously, *B. subtilis* PTS-394 was tested in tomato against gray mold disease and several defense related genes such as NPR1 and PR-1a were expressed after 72 h (Qiao et al. 2017). But the exact mechanisms of this endophytic strain L1-21 in tomato to express similar response needs to be studied further and is not known yet.

Tomato (Solanum lycopersicum L.) is the most broadly cultured vegetable globally which is susceptible during cultivation and this plant is susceptible to pathogenic fungi causing losses in crop yields up to 78% (Chavan and Borkar, 2020), in which *Botrytis cinerea Pers.* causing grey

mold disease, is a highly damaging necrotrophic plant pathogen, mainly affecting fresh fruits and vegetables. This fungus can invade different plant parts such as stems, leaves, flowers, fruits, and seeds before and after harvesting, leading to significant crop losses (Hua et al. 2018). This pathogen enters the host through pruning cuts, leaves, and flowers in greenhouse (Droby et al. 2007).

Many fungicides are used to control *B. cinerea* , but this management is not method considered sustainable due to their harmful effects on human health and the environment. Also, their frequent use increases the risk of developing resistance in pathogenic fungi, which is an important limiting factor in chemical control of *B. cinerea* . Therefore, finding non-chemical techniques like biological control as a viable and promising alternative method has been considered. But, the number of biological fungicides available at the market shelves effective against *B. cinerea* is limited (Haidar et al., 2016).

Consequently, there is a necessity to explore the novel microorganisms or their metabolites with the ability to combat *B. cinerea.* This quest for new control measures against the phytopathogens has prompted the utilization of endophytes as biocontrol agents. Endophytic fungi are organisms that can settle down inside the plants without any disease symptoms. They hold significant influence on host plants by enhancing plant tolerance to both biotic and abiotic stresses (Jia et al. 2016 and Aleahmad, 2023). Endophytic fungi have been isolated from various plant species, with some showing efficacy in suppressing *B. cinerea* (Bolívar et al. 2020).

Review of Literature and Development in the Subject.

During past two decades (Zhong et al. 2019). Adverse effects of biotic and abiotic factors have been negatively affecting tomato production gradually. Tomato crops are attacked by fungal pathogens that are considered the main contributors towards yield losses (Steinberg et al. 2020). *Botrytis cinerea* is a ubiquitous pathogen, develops gray mold symptom which is the most serious postharvest fungal disease in tomato plants. Gray mold causes rotting or decaying of fruit tissue during storage and commercialization. The postharvest losses in developed countries account for 15% – 20% of the total yield and even higher in developing countries up to 50% mainly due to poor preventive measures and storage conditions (Conti et al. 2019 and Nunes, 2012). Different factors that causes decay loses range from growing to handling at retail level. Transmitting bacteria before harvest are the most important issue (Bryden, 2012) mechanical injury in the form of fruits splitting, superficial grazing, bruising, crushing, deformation and rushing caused by inappropriate harvesting and transportation practices (Montero et al. 2009 and Opara, 2007 and Polat et al. 2012). In addition, improper processing, storage, packaging or marketing are the other important factors (Bendinelli et al. 2020). However, the fungal diseases are the main cause of postharvest losses (Carmona et al. 2019). Preventive measures should be considered for postharvest diseases in order to improve the fruit quality. The rotten fruits have potential hazards for example: the deterioration in fruit quality leads to reduced economic value; decayed fruits produce a series of metabolites or pathogenic microorganisms which produce various toxins, such as aflatoxins, zeaxanthin, vomiting toxins and hertlatoxin during storage (Wu et al. 2013). These toxins are

highly carcinogenic, teratogenic, and harmful to human and animal health (Collemare et al. 2019 and Liu et al. 2019).

In the last decades, it has been demonstrated that several beneficial EF can be artificially introduced on tomato using different inoculation methods and numerous protocols have been developed to successfully achieve this colonization, as well as to detect the fungi within the plant tissues. The methods used for the introduction and detection of EF in tomato plants are summarize. The inoculation of EF to tomato plants is mainly achieved with conidial suspension applied by seed treatments, root dipping, soil watering, stem injection, and leaf spraying. Alternatively, the application can be performed by mixing fungal biomass with the transplanting soil. Among the different inoculation techniques, the soil applications, mainly by watering with a conidia suspension, was the most commonly and successfully used technique applied in 18 studies. This was followed by the treatment of seed, as adopted in 15 studies, which was performed by various methods including seed soaking, seed coating, and seed dressing. Seed soaking consisted of placing the tomato seeds in a liquid conidial suspension for 2 to 24 h, before planting. Seed coating involved immerging the seeds in a conidial suspension, stirring them every 30 min for 2–3 h, to cover and adhere the spores to the seed surface, then successively air-drying under sterile conditions [93,94,113].

Seed dressing, was the less common technique, preparing and mixing the seeds in a conidia suspension with continuous shaking for several hours [74,103]. The conidia suspension usually contained a “sticker” such as Tween 80 (0.1–0.01% v/v) or methylcellulose (5–10% v/v), to ensure a more efficient adhesion of the conidia to the seed surface. Root dipping was another technique commonly used that consisted of dipping the seedling

roots in a conidial or propagule suspension for 6 to 24 h prior to transplant [71,76,79,89,106,110]. It is evident that the methods of application were numerous, and the selection of the most efficient method is highly dependent on the specific EF that is employed. The majority of the studies, involving the artificial introduction of EF to tomato, were conducted in a controlled environment, usually with sterilized soil or transplanting substrate, and not in the open field, in order to facilitate the monitoring of the plant colonization.

Aims & objectives of Research.

1. Selection & collection of different varieties of tomato plant for isolation of endophytic fungi.
2. Isolation of endophytes & causative agent of grey mold disease.
3. Microbiological study of endophytic fungi & causative agent of grey mold disease.
4. Molecular characterization of bioactive compounds from endophytic fungi.
5. Identification and molecular characterization, of endophytic fungi & causative agent of grey mold of *Solanum lycopesicum L*. (Tomato).
6. Study of growth promotion of tomato by endophytic fungi.

Methodology to be Adopted

1. Selection of different varieties of tomato plants.
2. From the selected varieties of tomato plants, the endophytic fungi and causative agent of grey mold disease of tomato to be isolated.
3. Microbiological study and study of growth promotion of tomato plant.
    - Identification and characterization of endophytic fungi.
    - Morphological study.
    - Biochemical study.
    - Study of growth promotion of tomato by selected endophytic fungus.
4. Molecular characterization of Bioactive compounds from endophytic fungi.
5. Control of 'Grey mold disease' pathogen by selected endophytic fungi.
6. Study of growth promotion of tomato by endophytic fungi.

Importance of Study/ Society application:

International Status:

The international research community has shown significant interest in utilizing endophytic fungi for the biological control of grey mold disease on tomatoes. Several countries, including China, India, Iran, and others, have conducted extensive research in this area.

Key Findings and Advances

* Diverse endophytic fungi: Researchers have isolated and identified a wide range of endophytic fungi from tomato plants, including species from genera such as Trichoderma, Penicillium, Fusarium, and Aspergillus.

* In vitro antagonism: Numerous studies have demonstrated the antagonistic potential of these endophytic fungi against botrytis cinerea in laboratory settings.

* In vivo biocontrol efficacy: Field trials have shown promising results, with endophytic fungi reducing disease severity and increasing crop yield.

* Mechanisms of action: The mechanisms of action of these fungi are diverse, including antibiosis, competition, and induced systemic resistance.

Future Outlook

The future of biological control of grey mold disease using endophytic fungi appears promising. Continued research is needed to:

* Identify and characterize novel endophytic fungi with strong biocontrol activity.

* Optimize the formulation and application of endophytic fungi-based products.

* Understand the complex interactions between endophytic fungi, plant hosts, and pathogens.

National Status :

India, as a major tomato-producing country, has been actively exploring sustainable and eco-friendly approaches to manage diseases like grey mold. Endophytic fungi have emerged as a promising biocontrol agent in this context.

Key Research and Developments:

* Isolation and Identification: Numerous studies have been conducted to isolate and identify endophytic fungi from various tomato cultivars grown in different agro-climatic zones of India.

* In Vitro Antagonism: Several endophytic fungal strains have been found to exhibit strong antagonistic activity against Botrytis cinerea in laboratory experiments.

* In Vivo Biocontrol Efficacy: Field trials have demonstrated the potential of these endophytic fungi to reduce disease incidence and severity in tomato crops.

* Mechanisms of Action: Researchers are investigating the mechanisms of action of these fungi, including antibiosis, competition, and induced systemic resistance.

Significance of the study

Using micro-organisms to control grey mold disease, also known has the potential to be a future control method. Bacillus genus most effective bacterial genera against phytopathogens. *Pseudomonas & paenibacillus* well studied in regard to botrytis biocontrol. Endophytes act as biocontrol

agents. They can Protect plants from pests & diseases by showing antagonistic activity against disease-causing phytopathogen.

They can degrade pollutants, which can improve environmental health. Endophytes are reported to produce a number of bioactive metabolite in a single plant or microbe which served as an excellent source of drugs for treatment against various diseases and potential applications in agriculture, medicine, food & cosmetics, industries. With the use of biofertilisers, healthy plants can be grown, while improving soil stability and health.

Proposed Work Plan:

Year wise work plan with targets to be achieved

First year

a. Course work
b. Selection and collection of different carieties of tomato plants.

Second Year

a. Isolation of endophytes and causative agent of grey mold disease.
b. Microbiological study and study of growth promotion of tomato plant

Third Year

a. Control of grey mold disease pathogen by selected endophytic fungi.
b. Molecular characterization of bioactive compounds from endophytic fungi.

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